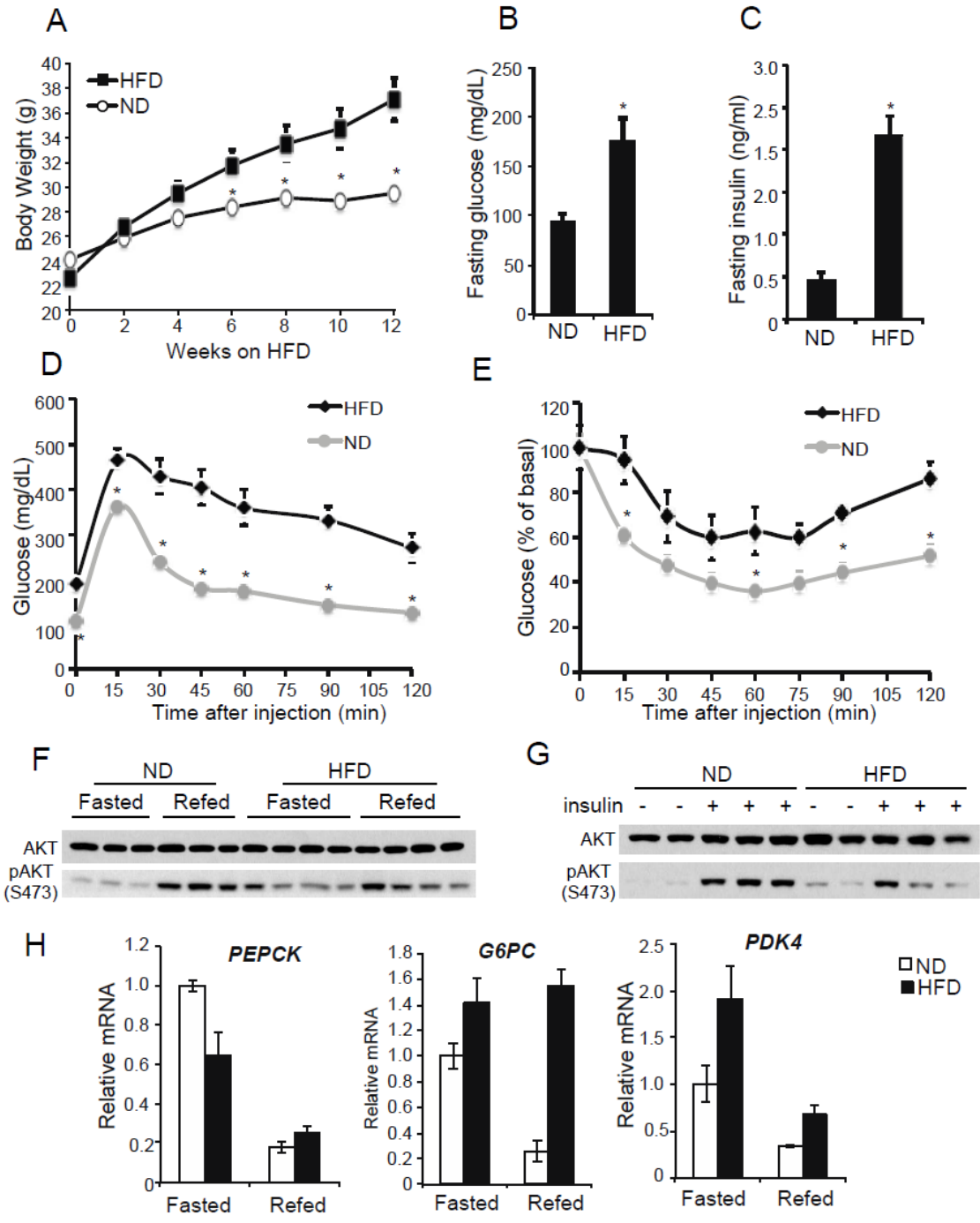
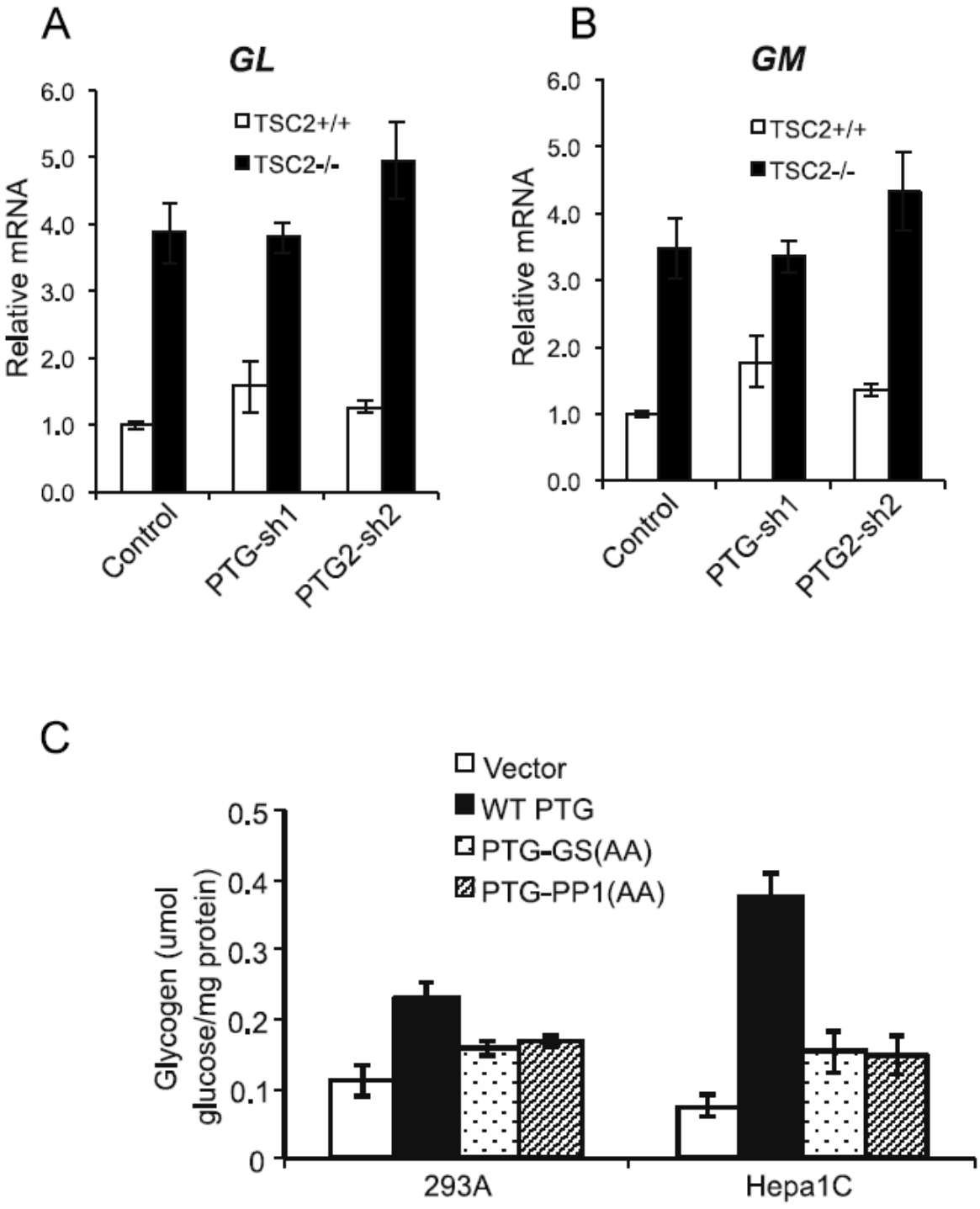
SUPPLEMENTARY DATA

Supplementary Figure 1. Metabolic phenotypes of C57BL/6J mice fed normal chow diet (ND) or high fat diet (HFD). Male mice aged 8-10 weeks were put on HFD for 12 weeks (n=8 each group). (A) Growth curve. (B) Fasting glucose level. (C) Fasting insulin level. (D) Glucose tolerance test. (E) Insulin tolerance test. (F) Representative Western blots showing pAKT (S473) levels from liver lysates of ND and HFD mice sacrificed after 16hrs fasting followed by 6hrs refeeding. (G) Representative Western blots showing pAKT (S473) levels from liver lysates of ND and HFD mice fasted overnight and sacrificed 20 min after intraperitoneal injections of insulin (2U/kg body weight). (H) Quantitative real-time PCR showing hepatic gluconeogenic gene expression. Values are expressed as mean ± SE.*indicates statistical significance by student t-test (*P<0.05).



SUPPLEMENTARY DATA

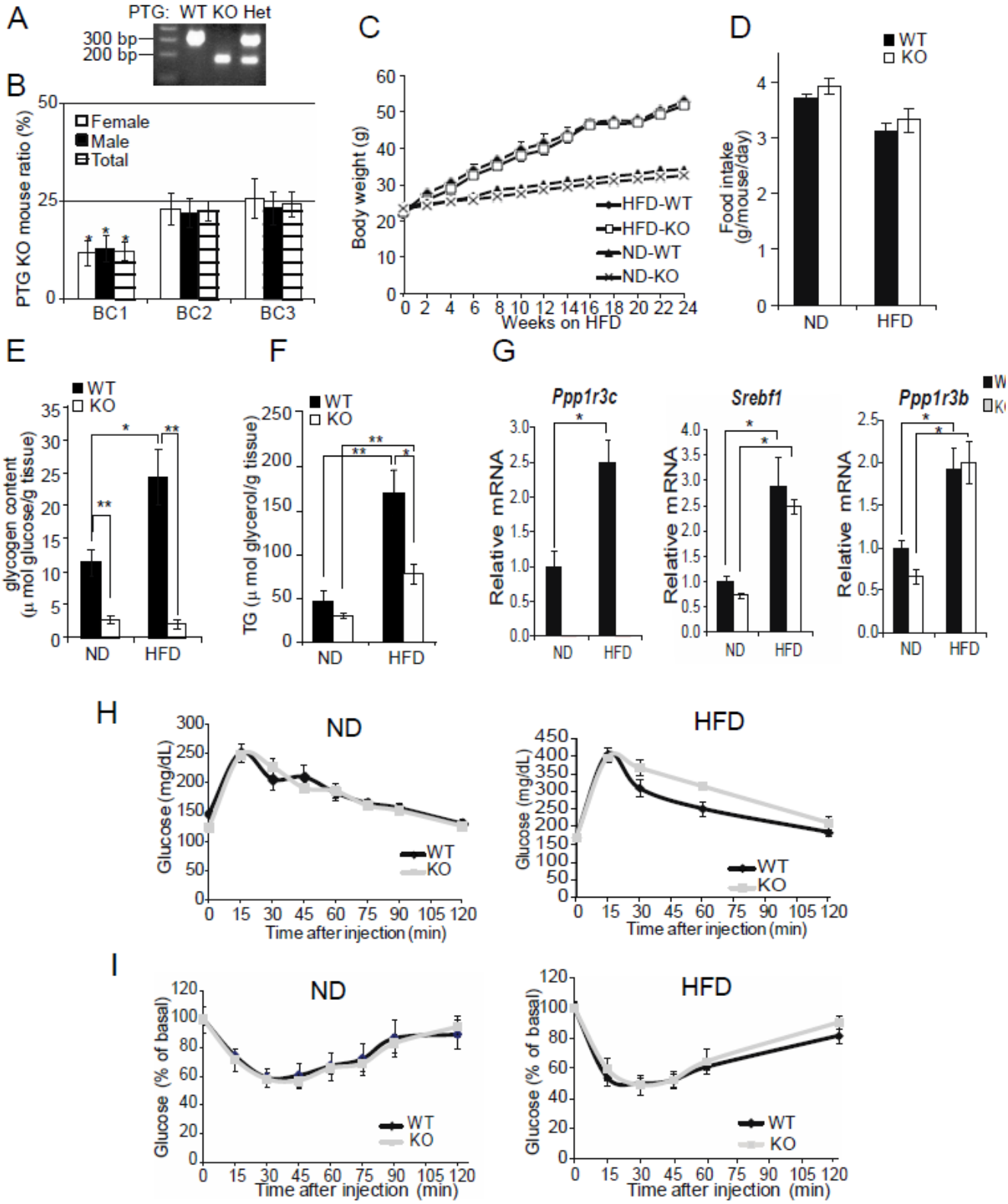
Supplementary Figure 2. Glycogen synthesis is similarly regulated in MEFs, 293 and liver cell lines. Quantitative real-time PCR showing GL (A) and GM (B) gene expression in TSC: PTG knockdown MEFs. (C) Glycogen content in 293A or Hepa1c cells transiently overexpressing WT PTG or PTG mutants incompetent to bind to GS or PP1.



SUPPLEMENTARY DATA

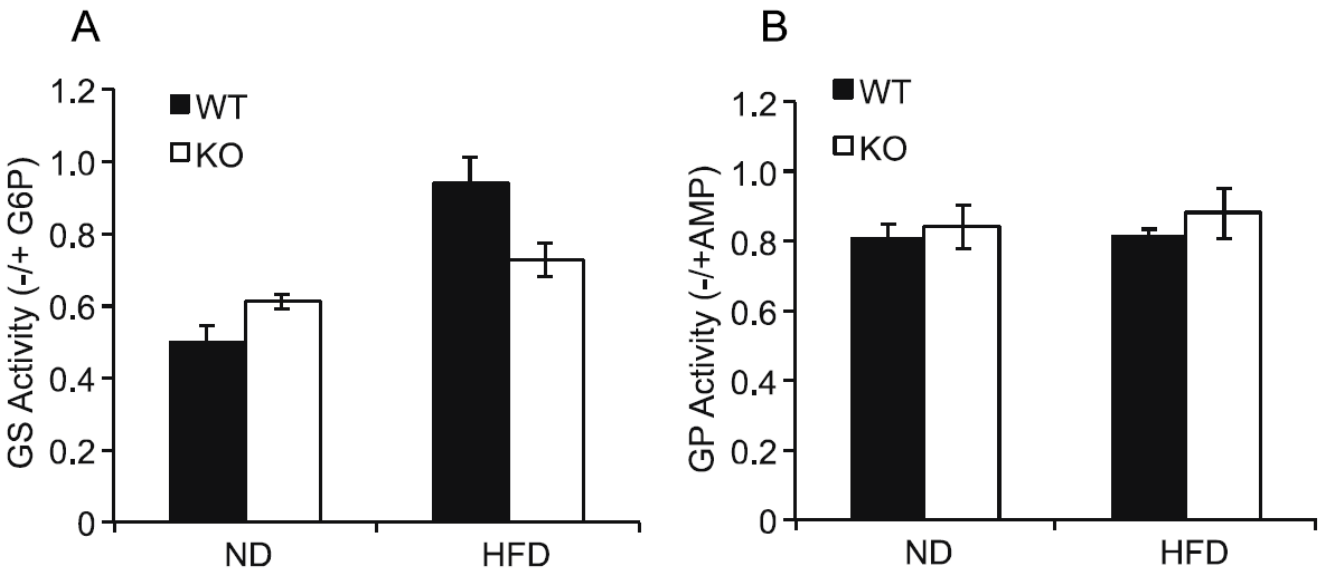
Supplementary Figure 3. PTG Ablation Results in Decreased Glycogen and Lipid levels in vivo after Short-term HFD without Changing Glucose and Insulin Sensitivity. (A) Genotyping of transgenic mouse showing homozygous deletion of the PTG gene. (B) PTG homozygous knockout mouse has improved survival rate at birth with more backcross. Heterozygous PTG knockout mice maintained on a mixed background were sequentially backcrossed with C57BL/6J mice and birth ratio of homozygous female, male, and total PTG knockout mice were calculated from genotyping of over 200 pups generated from heterozygous breeding pairs at each backcross generation. *indicates statistical significance by CHITEST relative to expected Mendelian ratio of 25% each group (*$P<0.05$). (C) Growth curves and (D) food intake for wild type (WT) or PTG knockout (KO) mice. Mice were fed a normal chow diet (ND) for 8 weeks before a high fat diet (HFD) feeding of 24 weeks. Body weights were measured every other week. Food intake was measured for consecutively three weeks (n=8-10 mice each group). (E) Glycogen content and (F) TG levels in the liver of WT and PTG KO mice on HFD for 12 weeks and fasted overnight (n=7-10 mice each group). (G) Quantitative real time PCR showing relative mRNA expression level of PTG (*Ppp1r3c*), SREBP1 (*Srebf1*) and GL (*Ppp1r3c*) in the same mice as in (E) and (F). (H) Oral glucose tolerance test and (I) Insulin tolerance test of WT and PTG KO mice on HFD for 12 weeks.

SUPPLEMENTARY DATA



SUPPLEMENTARY DATA

Supplementary Figure 4. GS and GP activity is not changed in PTG KO mice. (A) Hepatic GS activity (-/+ G6P) and (B) GP activity (-/+AMP) from ND and HFD PTG mice sacrificed after 16hrs fasting (n=8-10 mice each group).



SUPPLEMENTARY DATA

Supplementary Table 1. List of Real Time PCR Primers.

Name	Gene Symbol	Primer Sequence
FAS	*Fasn*	F: 5'-GGAGGTGGTGATAGCCGGTAT-3' R: 5'-TGGGTAATCCATAGAGCCCAG-3'
HMGCR	*Hmgcr*	F: 5'- AGCTTGCCCGAATTGTATGTG-3' R: 5'-TCTGTTGTGAACCATGTGACTTC -3'
LDLR	*Ldlr*	F: 5'- AGTGGCCCCGAATCATTGAC-3' R: 5'-CTAACTAAACACCAGACAGAGGC -3'
GM	*Ppp1r3a*	F: 5'-GCTTCCCGGAGAGTTTCCTTT-3' R: 5'-CACGGCTTTCTGGACTTGGA-3'
GL	*Ppp1r3b*	F: 5'-GTGGACATCCAATACAGCTACAG-3' R: 5'- CCGAGAACACTTTCACCATTGT-3'
PTG	*Ppp1r3c*	F: 5'-TGATCCATGTGCTAGATCCACG-3' R: 5'-ACTCTGCGATTTGGCTTCCTG-3'
GS (liver)	*GYS2*	F: 5'- ACCAAGGCCAAAACGACAG-3' R: 5'-GGGCTCACATTGTTCTACTTGA-3'
RPLP0-1[*]	*Rplp0*	F: 5'-CACTGGTCTAGGACCCGAGAA-3' R: 5'-AGGGGGAGATGTTCAGCATGT-3'
RPLP0-2	*Rplp0*	F: 5'-GAAACTGCTGCCTCACATCCG-3' R: 5'-GCTGGCACAGTGACCTCACACG-3'
SREBP1	*Srebf1*	F: 5'-AGGCCATCGACTACATCCG-3' R: 5'-TCCATAGACACATCTGTGCCTC-3'
G6PC	*G6pc*	F: 5'-CGACTCGCTATCTCCAAGTGA-3' R: 5'-GTTGAACCAGTCTCCGACCA-3'
PEPCK	*Pck1*	F: 5'-CTGCATAACGGTCTGGACTTC-3' R: 5'-CAGCAACTGCCCGTACTCC-3'
PDK4	*Pdk4*	F: 5'-GGAGTGTTCACTAAGCGGTCA-3' R: 5'-AGGGAGGTCGAGCTGTTCTC-3'
PKLR	*Pklr*	5'-TCAAGGCAGGGATGAACATTG-3' 5'-CACGGGTCTGTAGCTGAGTG-3'
GCK	*Gck*	F: 5'-ATGGCTGTGGATACTACAAGGA-3' R: 5'-TTCAGGCCACGGTCCATCT-3'
TNFa	*Tnf*	F: 5'-ACGGCATGGATCTCAAAGAC-3' R: 5'-AGATAGCAAATCGGCTGACG-3'
F4/80	*Emr1*	F: 5'-CTGGGATCCTACAGCTGCTC-3' R: 5'-AGGAGCCTGGTACATTGGTG-3'
CD11C	*Itgax*	F: 5'-CTGGATAGCCTTTCTTCTGCTG-3' R: 5'-GCACACTGTGTCCGAACTCA-3'
Rantes	*Ccl5*	F: 5'-GCTGCTTTGCCTACCTCTCC-3' R: 5'-TCGAGTGACAAACACGACTGC-3'
MCP-1	*Ccl2*	F: 5'-TTAAAAACCTGGATCGGAACCAA-3' R: 5'-GCATTAGCTTCAGATTTACGGGT-3'

[*]Triplicates of each sample were prepared and normalized to *RPLP0* (primer1) to determine relative expression levels. In the case of TSC2 knockout MEFs, we observed significant changes in the levels using this primer so we used *RPLP0* (primer2) for normalization.

SUPPLEMENTARY DATA

Supplementary Table 2. List of Genes Shown on the Heatmap from Figure 8C.

Gene Symbols
Cyp7a1
Hmox1
Aco2
Apoa2
Mttp
Apoa5
Elovl3
Pal2g6
Irs2
Tm7sf2
Nr0b2
Pck1
Fdps
Insig1
Aldoc
Idi1
Sqle
Acss2
Acat2
Ebp
Fads1
Idh1
Dbi
Pmvk
Pgd
Ggps1
Pcyt1a
Ucp2
Acly
Ldlr
Fasn
Nss
Nsdhl
Mid1ip1
Cyb5b
Hsd17b7
Dhcr24
Fdft1
Tmem97
Rdh11
Srebf2
Dhcr7
Mvk
Thrsp
Mmab
Hmgcr
Pfkfb1

SUPPLEMENTARY DATA

Supplementary Method

Enzyme activity assay. GS activity was determined by measuring incorporation of 14C-glucose from UDP- 14C-glucose into glycogen in the absence or presence of G6P as described previously (5). GP activity was assayed by measuring incorporation of 14C-glucose from 14C-glucose-1-phosphate into glycogen in the absence or presence of AMP (6). Activity ratios represent the activity measured in the absence of the allosteric effector (G6P for GS or AMP for GP) divided by that in the presence of the allosteric effector.

Primary hepatocyte isolation. Primary hepatocyte isolation and cultures were carried out as previously described (7). Cells were starved in serum-free media for 3 hr prior to the incubation with 14C-glucose for 1hr. Glucose conversion into glycogen, lipid and CO2 assay was performed as described in (8,9). Values are normalized to the protein content of hepatocytes and averaged from three independent experiments using primary hepatocytes isolated from 3 WT and 4 PTG KO mice on ND individually.

Euglycemic hyperinsulinemic clamp experiments. Euglycemic hyperinsulinemic studies were performed by the Animal Phenotyping Core of the Nutrition Obesity Research Center from University of Michigan, as previously described (10). Liver glycogen and lipids were extracted and 3H counts were calculated for *in vivo* glucose incorporation experiment.

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